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**Detection of Catabolic Genes in Environmental Samples from a
Polycyclic Aromatic Hydrocarbon-Contaminated Creek Using
Polymerase Chain Reaction**

A Senior Honors Thesis

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Microbiology 402
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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic compounds made up of two or more aromatic rings. In nature, these compounds pose problems because they are normally thermodynamically stable and not readily degraded. In addition, PAHs pose potential health risks to humans, as they can be toxic, mutagenic, and carcinogenic. Several microbial species contain genes that encode catabolic dioxygenases and monooxygenase enzymes, which are responsible for the initial step in the biodegradation of PAHs. In this study, polymerase chain reaction (PCR) was used as a molecular tool for the detection of these genes in environmental samples taken from the PAH-contaminated Chattanooga Creek in Chattanooga, TN. Nine primers sets, five designed for this study and four designed in study done by Baldwin et al. (2003), were used. These primers were based on the gene sequences of the large subunit of the dioxygenases or monooxygenases. We found the presence of one gene, *nidA*, found in the genome of several *Mycobacterium* sp., which encodes the large subunit of a dioxygenase capable of degrading high molecular weight (HMW) PAHs such as pyrene and benzo[a]pyrene.

INTRODUCTION

Polyaromatic hydrocarbons (PAHs) are ubiquitous organic compounds consisting of two or more aromatic rings. PAHs made up of more than three aromatic rings are considered high molecular weight (HMW) PAHs. PAHs are introduced into the environment through disposal of coal processing wastes, petroleum sludge, asphalt, creosote, and other wood preservative wastes (Habe et al. 2003). Because of their complex structure, these compounds are not readily degraded and can persist in the environment for many years. PAHs are of much concern because they can be toxic, mutagenic, or carcinogenic and are hazardous to human health. The Environmental Protective Agency (EPA) has identified 16 PAHs as priority pollutants (Habe et al. 2003; Khan et al. 2001). Of these 16 PAHs, this study focused on the biodegradation of pyrene, benzo[a]pyrene, naphthalene, and phenanthrene.

There have been many studies concerning the biodegradation of PAHs. Many microbial species that have been identified can degrade PAHs. The catabolic enzymes involved in the initial attack on PAHs are multisubunit oxygenases that introduce molecular oxygen, beginning the cascade of cleavages that eventually yield tricarboxylic acid cycle (TCA) intermediates. Because the gene sequences of many of these enzymes has been identified, molecular tools can be used to detect them in a culture or environmental sample. In this study polymerase chain reaction (PCR) was used a detection method and the primers were used to target the following genes: *nidA*, *clcR*, *narAC*, *phnAC1*, *todC1*, *bedC1*, *tmo*, *xyl*, *xylM*, *ntnM*, *nahAc*. Each of these genes encodes for the large subunit of a dioxygenase or monooxygenase, which catalyze the initial step of biodegradation.

The polymerase chain reaction works by replicating a given sequence many, many times by cycling through three steps. The first step is a 95°C denaturation, in which the double-stranded DNA is separated into two strands. During the second step, the primers anneal to the strands of DNA at the complimentary sequences. The final step, elongation, involves the elongation of the chain downstream of the primers by the polymerase, making two copies of the sequence.

There are have been several studies conducted in which PCR was used as a molecular tool for detection of certain genes. In a study done by Cavalca et al. (2004), PCR amplification was used to detect the *tmo* gene from species of *Pseudomonas*,

Microbacterium, *Azoarcus*, *Mycobacterium*, and *Bradyrhizobium* from groundwater. Moser et al. (2001) successfully used the large (α) subunit genes of the initial PAH dioxygenases as targets for PCR detection because the genes are highly conserved in *Pseudomonas*, *Comamonas*, and *Rhodococcus*. A study done by Laurie et al. (2000) used PCR to detect the *phn* and *nah* genes in *Burkholderia* species from contaminated soil. In study researching the biodegradation of HMW PAHs, Brezna et al. (2003) used PCR to detect the *nidA* and *nidB* gene in *Mycobacterium* species.

Several new primer sets were developed for this study. The *nidA* gene is found in the genome of several strains of *Mycobacterium* species. The primers were based on the sequences of five of these strains, including PYR-1, PYR-GCK, *M. frederiksbergense*, and *M. gilvum*. The gene encodes a dioxygenase that is able to degrade HMW PAHs such as pyrene and benzo[a]pyrene (Krivobok et al. 2003; Brezna et al. 2003; Habe et al. 2003). In one of the proposed catabolic pathways for the biodegradation of pyrene, the four-ringed PAH is initially attacked by the dioxygenase of the *Mycobacterium* species. After several steps, the compound is broken down into *o*-phthalic acid (Krivobok et al. 2003). Another proposed pathway shows cinnamic acid as the product of catabolism (Habe et al. 2003).

The primers used to target the *clcR* gene were designed based on sequences from *Ralstonia* and *Pseudomonas* species. This gene is a mobile degradative element involved in the biodegradation of chlorocatechol and biphenyl (van der Meer et al. 2001). Although these compounds are not PAHs themselves, they are often intermediates in the catabolic breakdown of chlorinated PAHs.

The sequences of several *Rhodococcus* species were used to design the primers targeting the *nar* gene. This gene encodes the large and small subunits of *cis*-naphthalene 1,2-dioxygenase (Andreoni et al. 2000). This dioxygenase, specific to *Rhodococcus*, has been shown to be a part of the catabolism of naphthalene into *cis*-naphthalene dihydrodiol (Larkin et al. 1999).

The sequences of the *phnACI* gene from species of *Burkholderia* and *Herbaspirillum* were used to design primers targeting this gene. Encoding the iron sulfur protein large subunit of PAH dioxygenase, this gene encodes an enzyme that is responsible for the catabolism of naphthalene as well as phenanthrene (Kasai et al. 2003).

The *nahAc* gene of γ -proteobacter, encoding the large subunit of naphthalene dioxygenase, was also gene target in this study.

The genes *todC1*, *bedC1*, *tmo*, *xyl*, *xylM*, and *ntnM* were targeted by primers designed in another study (Baldwin et al. 2003). The genes *todC1* and *bedC1* encode toluene/benzene/chlorobenzene dioxygenases. The *tmo* gene encodes a ring hydroxylating monooxygenase. The *xyl*, *xylM*, and *ntnM* genes encode a toluene monooxygenase (Baldwin et al. 2003).

This study was designed to test the hypothesis that PCR can be used as a method of detection of genes encoding for the various oxygenase enzymes in the environment. According to Baldwin et al. (2003), PCR primer sets targeting the large subunit of aromatic oxygenases could be used to detect catabolic pathways present in cultured organisms. The large subunit of the genes was used because of their substrate specificity and because it can be a rate limiting step in PAH biodegradation (Baldwin et al. 2003). Unlike the Baldwin et al. (2003) study, this study used environmental samples instead of pure cultures in the lab. The objective of this study is to use PCR as a molecular tool to detect catabolic genes present in microbial communities actively degrading PAHs at the heavily contaminated Chattanooga Creek Superfund Site. This study is unique in applying a comprehensive set of primers for detection of genes in sediments, suspended particles and flood plain communities at this site. In addition, this study also aims to test previously published primer sets designed based on pure cultures (Baldwin et al. 2003) on environmental microbial communities.

MATERIALS AND METHODS

Site Description

Chattanooga Creek, located in Chattanooga, TN has historically been a site of heavy industry, including the manufacturing of coke, organic chemicals, wood preservatives, and leather products. Between 1918 and 1987, the Tennessee Products Coke Plant, a coal carbonization facility, used the nearby Chattanooga Creek as dumpsite for coal tar wastes. As a result, the creek sediments were heavily contaminated with polycyclic aromatic hydrocarbons (PAHs). Placed on the National Priority List in 1995, a 2.5 mile section of the creek as been designated a Superfund site by the EPA. Although remediated in 1998, PAH concentrations remain high (Dionisi et al. 2004).

Field Sampling

All of the samples used in this study were collected from different locations along Chattanooga Creek in Chattanooga, TN (Figure 1). This creek has been designated a superfund site by the EPA due to heavy contamination with coal tar and associated organic contaminants, including polycyclic aromatic hydrocarbons (PAHs). Samples were collected from the creek sediment and water. Water samples were filtered through polycarbonate filters (pore sizes 5 and 10 μM) to collect the suspended sediments. After the samples were collected, they were placed in Zip-Loc bags, placed in a cooler with dry ice, and transported back to the lab in Knoxville.

DNA Extraction

Upon returning to the lab in Knoxville, the samples were allowed to thaw. The DNA was extracted from duplicate 0.5 g soil samples using the FastDNA Spin Kit (QBio-gene) and using the manufacturer's protocol. To lyse the bacterial cells and release the DNA, the samples were placed in the FastPrep FP 120 (Bio101 Savant) at a speed of 6.0 for 30 seconds. This was repeated twice. After extraction, the DNA samples were quantified using a pico-green assay (Molecular Probes) on the Victor² 1420 Multilabel Counter (Wallac).

Primer Design

Primer sets designed for this study targeted conserved regions of the respective gene based on alignments of gene sequences deposited in NCBI's GenBank from both cultured and uncultured organisms. As >99% of environmental microorganisms are thought to be unculturable, we include the sequences of uncultured organisms in attempt to improve the specificity of our primers. All alignments were done using either BioEdit Sequence Alignment Editor software (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) or EBI's ClustalW (<http://www.ebi.ac.uk/clustalw/>) to determine conserved sequences. Primers were then manually designed at conserved regions, using the Net Primer web-based software tool (<http://www.premierbiosoft.com/netprimer/netprimer.html>) to check oligonucleotide properties (melting temperature and GC content) as well as potential for hairpin or dimer formation. Primers were synthesized by Sigma-Genosys (The Woodlands, TX). Sequences and properties of the primers used are listed in Table 1.

NidA fw and rv primers were designed to amplify the pyrene dioxygenase genes from *Mycobacterium* sp. PYR-1 (AF249301), *M. flavescens* strain PYR-GCK (AF548343), *M. frederiksbergense* (AF548345), *M. sp.* 6PYR1 (AJ494745), *M. gilvum* strain BB1 (AF548347) and uncultured clones (AY032938 – AY032942).

ClcR fw and rv primers were designed to amplify the chlorocatechol dioxygenase genes from *Pseudomonas aeruginosa* (AF164958 and AF087482), and *Ralstonia* sp. JS705 (AJ006307).

RhoNar fw and rv were designed to target the naphthalene dioxygenase (*narAc*) genes from high GC gram positive organisms, based on the sequences of *Rhodococcus* sp. strains (AY392423, AY392424, AF121905, AB110633, AF082663, AJ401612, and AB024936).

PhnAc fw and rv primers were designed to amplify the naphthalene dioxygenase genes from *Burkholderia* spp. strain RP007 (AF061751), *B. phenazinium* (AY154365, AY154362, AY154361, and AY154364), *B. glathei* (AY154360 and AY154358), *Herbaspirillum* sp. Hg1 (AY154358), *Ralstonia* sp. PJ531 (AB066446), *Delftia*

acidovorans (AY367789) and uncultured clones (AY032935, AY032936, and AY032937).

ISPgamma fw and rv primers were designed to amplify the *nahAC* (naphthalene dioxygenase) genes from γ -proteobacter, including: *Pseudomonas putida* strains (AF306440, AF004284, AF306440, AF306430, AF306441, AF010471, NC_004999, and AF306432), *P. fluorescens* (AY048759 and AY433939) *P. stutzeri* (AF306427.1, AF039533, and AY196827), *P. aeruginosa* (AF448047), *P. balearica* (AF306420), as well as other pseudomonad strains (AY034588.1, AF306426, AJ496392, AJ496395, AF306433, NC_005244, AF306434, AF448050, and AF448052).

Table 1. Primers used in this study.

Primer Names	Target	Sequences (5' → 3')	Tm (°C)	Amplicon (bp)	Designer
NidA fw NidA rv	NidA	TTCCCGAGTACGAGGGATAC TCACGTTGATGAACGACAAA	59 59	141	This study
ClcR fw ClcR rv	ClcR	GGCAGCTTCGCTATTTTCATC CTGTATTTGGCGGGTAATGG	60 60	101	This study
RhoNar f RhoNar r	Nar	ATCCGCGGKACCTCAA GACTTCTTCAGCTGATTGC	51 49	243	This study
PhnAC f PhnAC r	PhnAC1	CCGGCATGGCAGGGAATAA CGATAAAGCCGGGCACGTAT	53 54	185	This study
B-tod fw B-tod rv	Tod C1, BedC1	ACCGATGARGAYCTGTACC CTTCGGTCMAGTAGCTGGTG	53	757	Baldwin et al. 2003
B-tol fw B-tol rv	Tmo, xyl	TGAGGCTGAAACTTTACGTAGA CTCACCTGGAGTTGCGTAC	55	475	Baldwin et al. 2003
B-rmo fw B-rmo rv	XylM, ntnM	TCTCVAGCATYCAGACVGACCG TTKTCGATGATBACRTCCCA	53	466	Baldwin et al. 2003
B-rdeg fw B-rdeg rv	XylM, ntnM	TYTCVGCATHCARACVGAYGA TTDTCGRTRATBACRTCCCA	52	466	Baldwin et al. 2003
ISP- γ fw ISP- γ rv	ISP gene γ - proteobacter	ACGG(C/T)GAACTGCAGA GATGAA(C/G)CCATCCAAGC	59	115	This study
16S fw 16S rv	Bacterial 16S rDNA	ATGGCTGTCGTCAGCT ACGGGCGGTGTGTAC	57.7 58.9	337	Harms et al. 2003

PCR Reactions

For each polymerase chain reaction (PCR), PurTaq Ready-To-Go PCR beads (Amersham Biosciences) were used. Each PCR tube contained one PCR bead, 400 nM of forward primer, 400 nM of reverse primer, and 0-400 nM of DNA template. The appropriate amount of water was added to make a 25 uL reaction. Along with the reactions, a negative control was run, in which no template was added to the reaction. Since there was not a positive control available for these catabolic genes, 16S primers (1055 fw and 1392 rv), see Table 1, were used as a method control, ensuring that PCR was set up properly and reactions resulted in amplification. For each reaction, the following touchdown PCR protocol was used: a denaturation step at 94°C for five minutes, then 94°C for 30 seconds, then annealing at 50°C to 60°C, depending on the T_m of the primer, for 30 seconds, then elongation at 72°C for one minute. For each cycle, the annealing temperature was decreased by 0.5°C to 1°C, depending on the annealing temperature (T_m) of the primer, per cycle. These steps were repeated for a total of 20 cycles. After these cycles, denaturation was performed at 94°C for 30 seconds, annealing at 40°C for 30 seconds, and elongation at 72°C for one minute. These steps were repeated for a total of 10 cycles. There was a final elongation step at 72°C for 10 minutes. In some instances, primers with melting temperatures within 2°C of each other were run in the PCR machine together, with the greater of the melting temperatures being used as the annealing temperature in the first cycle of PCR.

Preparation of Gel

Each PCR product was analyzed using gel electrophoresis. A 1.5% agarose gel was prepared based on the size of the gel desired. 1X TBE was mixed with Agarose Low EEO (Fischer Scientific) powder and warmed in the microwave until all of the agarose was dissolved. 0.5% ethidium bromide was added to the mixture, and the mixture was poured in gel plate. The appropriate gel comb was used, and the mixture was allowed to solidify at room temperature.

Analysis of PCR Product

3.5 uL of loading dye was added to each PCR reaction. 20 uL of 1Kb+ DNA ladder (Invitrogen) was added to the first well of the gel. The total contents of each PCR

reaction were then loaded into the remaining wells. Depending on the size of the gel, each gel was run at a voltage of between 65 and 80 for approximately one hour. A picture of the gel was taken using the MultiImage Light Cabinet and AlphaImager v5.5 software (Alpha Innotech Corporation). The picture was carefully analyzed for the presence of an amplicon of the appropriate size according to the primer design (see Table 1).

Optimization of PCR

If a band of the appropriate size was present, a PCR temperature gradient was used in order to determine the optimal PCR conditions for the reaction. In cases where the bands were very faint even after running a temperature gradient, a template concentration gradient was also used. About midway through the project, the thermal cycler with gradient capabilities malfunctioned, and temperature gradients could no longer be run. Also, if there was a band, PCR was run using three negative controls, in order to be sure that there were no primer artifacts. These controls included a forward primer plus template reaction, a reverse primer with template reaction, and a forward and reverse primer with no template reaction. After optimal conditions were found and it was determined that there was no nonspecific annealing, the PCR reaction was run again.

Cloning of PCR product from the gel

After the PCR reaction was run again at optimal conditions, it was run on a gel. The gel box was covered with a piece of paper, to minimize exposure of DNA to UV light. After the gel was run, the gel was placed in the light cabinet, and UV light was applied just long enough for the location of the correct bands to be determined. The bands were then cut out with a clean cutting blade, and the mass of the gel slice measured. The DNA was extracted from the gel using the Qiagen MinElute Gel Extraction Kit.

After the gel extraction was complete, the DNA was cloned and transformed according to the protocol in the Invitrogen TOPO pCR 4.0 TA Cloning Kit. The transformants were plated on Luria Broth agar with 50 mg/mL kanamycin and incubated overnight at 37°C. All transformant colonies were transferred onto a grid plate. This grid plate was incubated overnight at 37°C.

Isolation of Plasmids

Transformants on the grid plate were grown in LB with 50 mg/mL kanamycin overnight at 37°C with shaking. The plasmid DNA was then isolated from each of the growth cultures using the Wizard Plus Minipreps DNA Purification System Kit and protocol. The isolated DNA was then quantified using the Hoefer DyNA Quant 200 Fluorometer (Amersham Biosciences).

The plasmids were digested with EcoRI to verify that there was an insert in the plasmid, and that insert was the correct size compared to the gene of interest. The digestion included a 20 uL mixture containing 0.5 uL of EcoRI (Promega), 2 uL of Buffer H, 15.5 uL of water, and 2 uL of template. A master mix was prepared containing the EcoRI, buffer and water. The DNA was digested overnight at 37°C, and then run on a gel. Samples containing the correct size insert were then sent for sequencing.

Cloning from the PCR product

When the band in the gel was too faint, or DNA extraction from the gel was unsuccessful, the PCR products were cloned immediately after being removed from PCR machine. The DNA samples were run at optimal conditions, and 2uL of the PCR product was used in the TOPO TA Cloning reaction.

Analysis of DNA Sequences

The plasmids were sequenced with the M13 primer (included in the TOPO kit) at the Molecular Biology Resource Facility, University of Tennessee, using an ABI 3100 automated capillary DNA sequencer. The DNA sequences were analyzed using the nucleotide-nucleotide BLAST (blastn) search from the NCBI website (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Long-term Storage of Positive Clones

If sequencing showed that the plasmid contained the correct gene, the corresponding colony from the grid plate was grown in LB/Kanamycin broth in a flask

overnight at 37°C with shaking. An aliquot of the broth plus 25% glycerol was placed in a cryovial and stored at -80°C.

RESULTS

Table 2. The following samples were screened with each of these primers. Sediment samples were collected from the creek bed. Filtered samples represent the suspended sediments. Flood plane soil samples were collected from the soil in the flood plane surrounding the creek. (✓) symbol represents a positive hit. (–) symbol represent no hits. (?) symbol represents a negative hit. (U) symbol represents unavailable data.

2A. Sediment Samples

Sample	Description	NidA ^a	ClcR ^b	RhoNar ^c	PhnAC ^d	Btod ^e	Btol ^f	Brmo ^g	Brdeg ^h	Isp-γ ⁱ
CF	Crabtree Farms (CF), June 2004	-	-	-	-	-	-	?	-	-
FM	FM, July 2004 (12" down)	-	-	-	-	-	-	-	-	U
FM	Sediment from FM, July 2004 (surface)	-	-	-	-	-	-	-	-	U
DO	Dock area, July 2004 (15" down)	✓	-	-	-	-	-	-	-	U
DO	Dock area, July 2004 (24" down)	-	-	-	-	-	-	-	-	U
FM	FM, July 2004 (upstream)	-	-	-	-	-	-	-	-	U

^a primer for the gene target *nidA*

^b primer for the gene target *clcR*

^c primer for the gene target *nar*

^d primer for the gene target *phnACI*

^e primer for the gene targets *todCI* and *bedCI*

^f primer for the gene targets *tmo* and *xyl*

^g primer for the gene targets *xylM* and *ntnM*

^h primer for the gene targets *xylM* and *ntnM*

ⁱ primer for the gene targets *nahAC*

SSWB	CF, June 2004									
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2C. Filtered Samples

Sample	Description	NidA	ClcR	RhoNar	PhnAC	Btod	Btol	Brmo	Brdeg	Isp- γ
CF flood	nearby swampy area, CF, June 2004	-	-	?	-	-	-	-	-	-
CF	Creek whole water from CF, June 2004	-	-	-	-	-	-	-	-	-
FM 1	FM, July 2004	-	-	?	-	-	-	-	-	U
FM 2	FM, July 2004	-	-	?	-	-	-	-	-	U

Several primer-sample combinations displayed amplification during initial PCR, but after testing with negative controls or cloning, these combinations were shown to not contain the correct gene. These combinations included phnAC-DO, phnAC-PF2, rhoNar-BT1, rhoNar-PF2, rhoNar-CF flood, rhoNar-FM 1, rhoNar-FM 2, and brmo-CF. The primer combinations that did display positive results included nidA-CF2 and nidA-DO.

The samples showing a positive hit with the phnAC primers (designed for this study), displayed a recurring trend of the negative primer only control containing an amplicon of the same size. For clarify the identity of the bands, the band corresponding to the correct size of the gene target was cut out of the gel, cloned and the plasmid isolated according to the procedure described above. The sequences of the plasmid inserts were only 22 base pairs and were directly correlated to the reverse primer. None of the sequences from this primer corresponded to the correct gene.

The amplicons for the samples screened with rhoNar (designed for this study) were not the expected size. The designed amplicon was 243 base pairs, however, when used on concentrated template (> 100 ng), the amplicon appeared to be about 101 to 110 base pairs. When the DNA samples were quantified and diluted to 10 ng/uL and PCR was

performed again under the same reaction conditions, there were no amplicons. Several different methods were applied in order to repeat the results obtained during the first PCR run. First, the concentration of the sample DNA was increased to 50 ng instead of 10 ng. This had no effect, and no amplicons were present. Second, a concentration gradient of one of the templates, PF2-1, was created with concentrations at 100 ng, 200 ng, 500 ng, and 1000 ng. None of the reactions yielded visible bands. Third, the samples were run using a standard PCR protocol instead of a touchdown PCR program. This protocol included a denaturation step at 95° for 10 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 48°C for 30 seconds, and elongation at 72°C for 30 seconds. These steps were repeated for 35 cycles, followed by a final elongation step at 72°C for 10 minutes. The annealing temperature of 48°C was chosen since it was 1°C less than the T_m for the reverse primer. No amplicons were present after this method either. Because the apparent amplicons in the first reaction were the wrong size, and because amplification could not be reproduced, it was concluded that the amplicon was likely a primer artifact.

For the CF-brmo (Baldwin et al., 2003) combination, a very faint amplicon was present for the initial PCR run, however, these results could not be repeated during subsequent runs. In order to repeat these results, the concentration of the template DNA was increased to 25 ng and 50 ng, but no amplicons were present. Next, the annealing temperature was decreased to 55°C -0.5°C/cycle using 10 ng and 25 ng of sample. These conditions did not yield any amplicons. Finally, the annealing temperature was decreased to 50°C, which is 3°C below the T_m , using a standard PCR protocol. No amplicons were observed under these conditions either.

The primers that yielded positive results during these experiments were the *nidA* primers. These primers target the *nidA* gene of *Mycobacterium flavescens* strain PYR-GCK which encodes the large subunit of a pyrene dioxygenase enzyme. This dioxygenase has been linked to mycobacterium's ability to degrade high molecular weight-PAHs such as pyrene and benzo[a]pyrene (Cheung et al., 2000). The samples that contained the *nidA* gene included CF2 and Center 15" down. The sequence of the gene was consistent among the clones (see Table 3)

Table 3. BLAST sequences with 100% similarity to the clones sequenced in the lab

Accession #	Species	Gene	Reference
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AY365117.2	<i>Mycobacterium vanbaalenii strain PYR-1</i>	<i>nidA</i>	Stingley et al. 2004
AF548345.1	<i>Mycobacterium frederiksbergense strain FAn9T</i>	<i>nidA</i>	Brezna et al. 2002
AF548343.1	<i>Mycobacterium flavescens strain PYR-GCK</i>	<i>nidA</i>	Brezna et al. 2002
AY330102.1	<i>Mycobacterium sp. MCS</i>	<i>nidA</i>	Miller et al. 2004
AY330100.1	<i>Mycobacterium sp. KMS</i>	<i>nidA</i>	Miller et al. 2004
AY330098.1	<i>Mycobacterium sp. JLS</i>	<i>nidA</i>	Miller et al. 2004
AF249301.2	<i>Mycobacterium sp. PYR-1</i>	<i>nidA</i>	Khan et al. 2001
AB179737.1	<i>Mycobacterium sp. MHP-1</i>	<i>nidA</i>	Habe et al. 2004

In summary, the sediment microbial community at Chattanooga Creek Superfund Site in Chattanooga, TN was shown to contain the *nidA* gene identical to that found in *Mycobacterium* sp. strain PYR-1, PYR-GCK, and others. This gene encodes an aromatic-ring hydroxylating dioxygenase involved in the initial step of biodegradation of HMW PAHs such as pyrene and benzo[a]pyrene. These findings suggest that PCR is an effective means of detecting catabolic genes in environmental samples. However, only one set of primers yielded successful amplification. The Baldwin et al. (2003) primer sets used in this study did not yield any substantial results, so it is unclear as to their effectiveness on environmental versus lab strains. Future experimentation should focus on designing primers that can cover the diverse gene sequences found in environmental samples.

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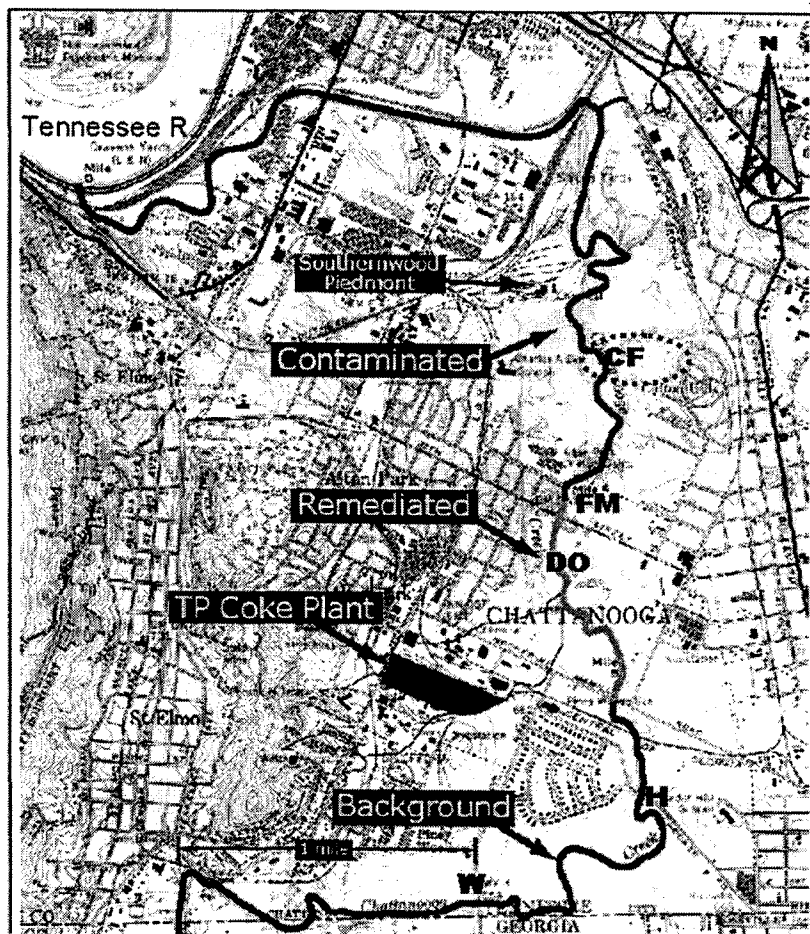


Figure 1. Sample sites along the Chattanooga Creek